SYNTHESIS OF CARBON-14 LABELED 4-BROMO-2,7-DIMETHOXY-3H-PHENOTHIAZIN-3-ONE

S. R. Prakash, R. L. Ellsworth and H. E. Mertel
Merck Sharp and Dohme Research Laboratories
Post Office Box 2000
Rahway, New Jersey 07065

SUMMARY

Two different syntheses of (5), one labeled with carbon-14 in the quinonoid ring and the other in the phenyl ring are described. Treatment of p-[U-^{14}C]benzoquinone with 2-amino-5-methoxy benzenethiol yielded (7), which was methoxylated to provide a mixture of isomers (4a) and (8). Separation of isomers followed by bromination of (4a) gave (5a). Thiocyanation of p-[U-^{14}C]anisidine provided (10), which was hydrolysed to give labeled aminothiol (3b); the latter was condensed with 2-bromo-6-methoxy-p-benzoquinone to afford (5b).

Key Words: P-[U-^{14}C]benzoquinone, P-[U-^{14}C]anisidine, 4-bromo-2,7-dimethoxy-3H-[U-1,2,3,4,4a,10a-^{14}C]phenothiazin-3-one, 4-bromo-2,7-dimethoxy-3H-[U-5a, 6,7,8, 9,9a-^{14}C]phenothiazin-3-one, leukotriene biosynthesis inhibitor.

INTRODUCTION

4-Bromo-2,7-dimethoxy-3H-phenothiazin-3-one (5) is an inhibitor of mammalian leukotriene biosynthesis and is thus a possibly useful therapeutic agent for treating conditions such as asthma, respiratory allergies, inflammation and certain skin diseases[1]. This compound is presently under development in our laboratories. As part of this ongoing program we prepared tracers for metabolism and disposition studies. In this report we describe two different routes of preparations, one involving labeling with carbon-14 in the quinonoid ring (5a) and the other with labeling in the phenyl ring (5b).

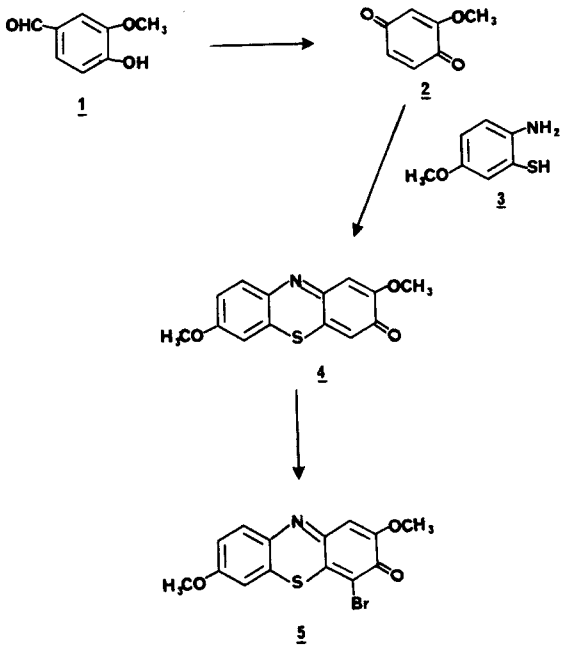
0362-4803/87/010097–09$05.00

Received March 19, 1986
Revised June 9, 1986

RESULTS AND DISCUSSION

The synthesis of (5) has been published[1]. As outlined in scheme I, 2-Methoxy-p-benzoquinone was obtained by Dakin oxidation of vanillin and then condensed with 2-amino-5-methoxybenzenethiol (3) to generate the phenothiazin skeleton.

Scheme I

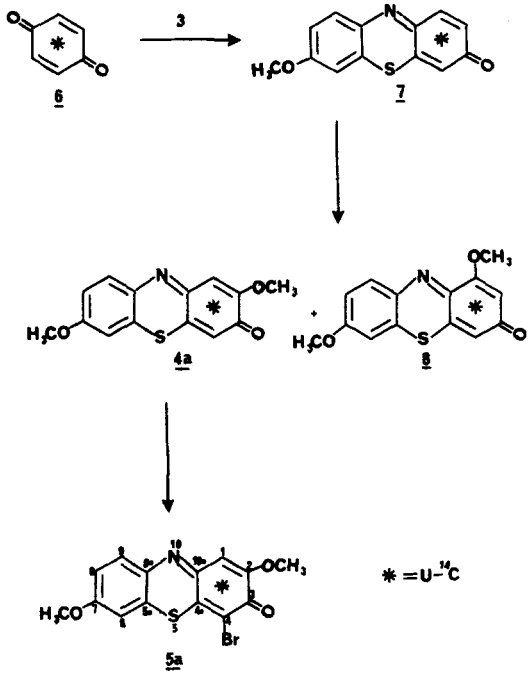


A direct adaptation of this route for the synthesis of the labeled compound would require [ring-^{14}C]vanillin. We chose to start with more readily obtainable p-[U-^{14}C]benzoquinone and use the route shown in scheme II to obtain (5a).

4-Bromo-2,7-dimethoxy-3H-[U-1,2,3,4,4a,10a-^{14}C]phenothiazin-3-one (5a)

Treatment of two equivalents of p-[U-^{14}C]benzoquinone with 2-amino-5-methoxybenzenethiol[2] in methanol afforded (7) in 41% chemical yield. The [U-^{14}C]hydroquinone produced during the reaction was reoxidized to benzoquinone with sodium metaperiodate and allowed to react with another portion of (3). Sodium iodate formed by the reaction of sodium metaperiodate and hydroquinone could itself act as an oxidizing agent and lead to the production of iodine. The crude product was therefore washed thoroughly with water and

Scheme II



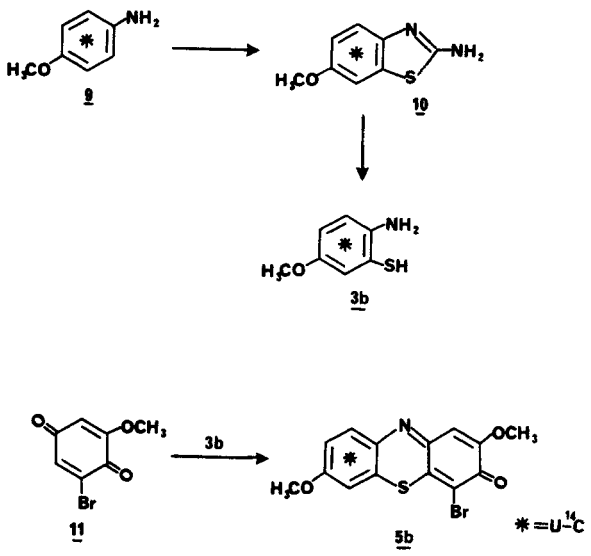
extracted with methylene chloride in order to free it from iodine and inorganic materials. The methylene chloride extract on concentration provided a second portion of (7). Interestingly, HPLC analysis of the product revealed the presence of an additional component [retention time similar to the desired (7)], the identity of which was established as (4a) by co-chromatography with an authentic unlabeled sample of (4a)[3].

Methoxylation of (7) was carried out in methylene chloride-methanol with potassium tert-butoxide. Trial experiments with unlabeled (7) had revealed that either sodium methoxide or potassium tert-butoxide in methanol could be used and that excess base was required. Use of sodium methoxide in THF resulted mainly in (8)[4]. The isomeric product mixture (4a) and (8) was separated by column chromatography on silica gel. Bromination of (4a) yielded (5a) in 85% chemical yield with radiochemical purity of 95%. Crystallization of the crude product from toluene-methanol afforded a sample of (5a) with radiochemical purity greater than 99%. The overall radiochemical yield from p-benzoquinone was 7.1%.

4-Bromo-2,7-dimethoxy-3H-[U-5a,6,7,8,9,9a-^{14}C]phenothiazin-3-one (5b)

For the synthesis of (5a) as shown in scheme II two equivalents of benzoquinone are nominally required for the condensation-reoxidation cycle. With p-[U-^{14}C]benzoquinone as the label carrier this inefficient utilization was mitigated somewhat by the aforedescribed recycling. A more effective use of label carrier was achieved by the alternate approach outlined in scheme III, in which p-[U-^{14}C]anisidine was employed as a label carrier, and which also permitted the use of 2-bromo-6-methoxy-p-benzoquinone (11), thus eliminating the previously encountered isomer problem. The resulting product was 4-bromo-2,7-dimethoxy-3H-[U-5a,6,7,8,9,9a-^{14}C]phenothiazin-3-one (5b).

Scheme III



Thiocyanation of p-[U-^{14}C]anisidine with ammonium thiocyanate and bromine in acetic acid resulted in the formation of 2-amino-6-methoxybenzothiazole (10) in 60% yield. 6-Substituted-2-aminobenzothiazoles have generally been prepared by the treatment of inorganic thiocyanates and bromine with para-substituted anilines[5]. We found it difficult to carry out the reaction as described in the literature[5]. The reaction solvent acetic acid freezes at 16°C and all published procedures recommend running the reaction at or below 10°C. We consistently obtained product yields of 60-65% by running the reaction at or slightly below 20°C. Alkaline hydrolysis of (10) led to (3b) in

87% yield. The aminothiol (3b) was condensed directly with nearly two equivalents of 2-bromo-6-methoxy-p-benzoquinone (11) to afford (5b) in 54% crude yield[6]. Crystallization of the crude product first from DMF and then toluene-methanol yielded tracer-quality material. The overall radiochemical yield from p-[U-^{14}C]anisidine was 14%.

EXPERIMENTAL

Radioactivity measurements were carried out using Packard Tricarb PLD and 3255 liquid scintillation spectrometers and Instagel ® (United Technologies). TLC analyses were carried out using Merck silica gel 60 F-254 plates and radiochromatogram scans were performed on a Berthold automatic TLC linear analyzer LB 2832. HPLC analyses were performed using Constametric pumps I and II, LDC UV detector at 280 nm, Hewlett Packard 3388A integrator and Berthold radioactivity monitor LB 503. The ^{1}H NMR spectra were recorded on Varian XL-200 and SC-300 spectrometers. Chemical shifts are expressed in parts per million downfield from internal tetramethylsilane. Mass spectra were taken on a LKB 9000 spectrometer. Methylene chloride was distilled from P_2O_5 and methanol was distilled from sodium prior to use. Para-[U-^{14}C]benzoquinone was obtained from the Amersham Corporation and para-[U-^{14}C]anisidine was obtained from Pathfinder Laboratories. Identities of the labeled compounds were established by co-chromatography with pure unlabeled compounds[1,8].

7-Methoxy-3H-[U-1,2,3,4,4a,10a^{14}C]phenothiazin-3-one (7)

To a suspension of p-[U-^{14}C]benzoquinone (43.5 mCi, 10.1 mCi/mmol) in methanol (7.6 ml) cooled to -40°C was added in one portion 2-amino-5-methoxybenzenethiol[2] (345 mg, 2.19 mmol) under nitrogen. The reaction mixture was allowed to warm to room temperature and then stirred for two hours. The mixture was filtered and the collected solid was washed with methanol (3 ml), ether (1 ml) and air dried to afford crude (7) (436 mg, Sp. act., 37.7 μCi/mg). HPLC: Whatman C8, 10 min gradient elution from 30% CH_3CN in water to 50% CH_3CN in water, 1 ml/min, rate of phosphor flow - 4 ml/min, retention time 12.4 min, radiochemical purity 92.8%. The mother liquor was concentrated to

about 10 ml, sodium metaperiodate (479 mg, 2.23 mmol) was added, and the mixture then stirred overnight. HPLC analysis indicated that less than 4% hydroquinone (retention time 3.5 min) remained. The mother liquor was cooled to-40°C and 2-amino-5-methoxybenzenethiol (233 mg, 1.5 mmol) was added in one portion under nitrogen. The mixture was allowed to warm to room temperature, stirred for 2 hours and filtered. The filter cake was washed with methanol (3 ml), water (20 ml) and then extracted with methylene chloride (53 ml). The methylene chloride extract was concentrated under a gentle stream of nitrogen to afford a second portion of crude (7) (245 mg, 6.0 mCi). HPLC analysis of this material showed radioactive (7) 57% with 22% of the radioactivity appearing in a peak with a retention time of 11.5 min. The identity of the additional peak was established using an unlabeled sample of (4). Co-chromatography (HPLC) of this second portion of crude (7) with a pure sample of (4) resulted in an increase of UV response of the peak with a retention time of 11.5 min and with virtually no effect on radiochromatograms.

4,7-Dimethoxy-3H-[U-1,2,3,4,4a,10a-^{14}C]phenothiazin-3-one (4a)

To a solution of crude (7) (436 mg, 16.43 mCi) in methylene chloride (25 ml) and methanol (22 ml) potassium tert-butoxide (1.34 g, 6.6 eq) was added and the mixture was stirred at ambient temperature. Aliquots of the reaction mixture were analyzed by HPLC. At the end of 7 days there remained less than 7% of (7). This reaction also generated two products more polar than (4a) or its isomer (8). The reaction mixture was diluted with water (110 ml) and extracted with methylene chloride (7 x 50 ml). The methylene chloride extract was dried ($MgSO_4$), filtered and evaporated under nitrogen to provide 296.3 mg (9.33 mCi) of the product mixture. The aqueous layer contained polar products (5 mCi)[7]. The crude product mixture was dissolved in *methylene* chloride and chromatographed on a column of silica gel (E. Merck silica gel 60 HR for TLC, 8 ml fractions). Elution with 1% methanol in methylene chloride provided 108 mg (3.29 mCi) of (4a) followed by 107 mg (2.89 mCi) of (8).

In a similar fashion the second portion of crude (7) (245 mg, 6 mCi) was dissolved in methylene chloride (15 ml) -methanol (15 ml) and treated with

potassium tert-butoxide (0.8 g). After 5 days of stirring at room temperature the reaction mixture was worked up as described above and chromatographed on silica gel to provide 55.6 mg (2.07 mCi) of (4a) followed by 33.5 mg (1.15 mCi) of (8).

4-Bromo-2,7-dimethoxy-3H-[U-1,2,3,4,4a,10a-^{14}C]phenothiazin-3-one(5a)

To a solution of (4a) (163 mg, 5.36 mCi) in methylene chloride was added acetic acid (4.9 ml). The methylene chloride was then evaporated under a gentle steam of nitrogen. To this suspension of (4a) in acetic acid was added a solution of bromine in acetic acid (2 ml, 100 mg/ml) over a 10 min period. The mixture was stirred at room temperature for 3 hours. Methanol (8 ml) was added and stirring was continued for an additional 1 hour whereupon the black suspension turned orange. The mixture was filtered and the collected solid was washed with methanol and air dried to afford crude (5a) (178 mg, 84.7%). Radiochemical purity of the crude product was 95% (TLC). Crystallization from toluene-methanol provided pure (5a) (110 mg, 3.19 mCi, 29 μCi/mg). The radiochemical purity of this material was greater than 99% by TLC/autoradiography (2% methanol in methylene chloride with 0.1 ml Conc NH_3) and HPLC (Whatman C8, 50% CH_3CN in water, 1 ml/min).

2-Amino-6-methoxybenzo[ring-^{14}C]thiazole (10)

To a solution of para-[U-^{14}C]anisidine (440 mg, 40.4 mCi, 11.31 mCi/mmol, 3.57 mmol) in acetic acid (6 ml) was added ammonium thiocyanate (558 mg, 7.33 mmol) and the mixture was stirred at room temperature for 30 min. The mixture was cooled to 15°C and a solution of bromine (1.145 g, 7.16 mmol) in acetic acid (0.9 ml) was added over a period of 3 hours. The internal temperature of the reaction mixture was kept below 20°C (17-20°C) by cooling in ice. The mixture was filtered and the product was washed with acetic acid (2 ml). The crude hydrobromide (1.28 g) was dissolved in warm water (25 ml), filtered, and the filtrate was basified with saturated solution of sodium carbonate. The precipitated product was filtered, washed well with water and dried *in vacuo* to provide (10) (366 mg, 24.8 mCi). The filtrate and the washings were extracted with ethyl acetate and the ethyl acetate extract was

concentrated to provide another portion of (10) (22.5 mg, 1.1 mCi). This compound was used in the next step without any further purification. HPLC: Whatman C8, 60% MeOH in water with 0.1% H_3PO_4, 1 ml/min, -radiochemical purity 95%; TLC: CH_2Cl_2-4% MeOH, -radiochemical purity 96%, ^1H NMR (200 MHz) ($CDCl_3$) 3.86 (S, 3H, OCH_3), 5.18 (br S, 2H, NH_2), 6.92, 7.16 and 7.48 (3H, aromatic Hs); MS, m/e 180,165,149,137,110.

4-Bromo-2,7-dimethoxy-3H-[U-5a,6,7,8,9,9a-^{14}C]phenothiazin-3-one (5b)

2-Amino-6-methoxybenzo[ring-^{14}C]thiazole (355 mg, 1.97 mmol, 23.6 mCi) was refluxed with 4 N potassium hydroxide solution (3 ml) under nitrogen overnight. The mixture was cooled, pH was adjusted to 10.5 by addition of conc. hydrochloric acid and then to pH 6 by 5N acetic acid causing the product to separate. The product was filtered, washed with water and dried *in vacuo* to provide (3b) (265 mg, 87%).

To a suspension of 2-bromo-6-methoxy-p-benzoquinone[6] (655 mg, 3.02 mmol) in methanol cooled to -40° was added a suspension of (3b) (265 mg) in methanol. The temperature of the reaction mixture was allowed to rise to room temperature slowly and then stirred at that temperature for 1 hour. The product was filtered, washed with methanol and dried to provide (5b) (324 mg, 54%, 10.8 mCi). HPLC analysis of this material indicated radiochemical purity to be nearly 80%. Two recrystallizations first from DMF and then toluene-methanol afforded (5b) (176 mg, 5.7 mCi, 32 μCi/mg) with radiochemical purity >99% by TLC and HPLC. ^1H NMR (300 MHz) ($CDCl_3$) 3.94 (S, 3H, OCH_3), 3.97 (S, 3H, OCH_3), 6.96 (S, 1H, C_1H), 7.16, 7.2 and 7.94 (3H, aromatic Hs); Ms, m/e 355,353,351,338, 324.

ACKNOWLEDGEMENT

We are grateful to Mr. H. Flynn for providing NMR spectra and to Dr. W.J.A. VandenHeuvel for providing mass spectra.

REFERENCES & NOTES

1. Guindon, Y., Fortin, R., Lau, C.K., Rokach, J. and Yoakim, C. - EP 115394; CA. 101: 230555t (1984).

2. (3) was freshly made from 2-amino-6-methoxybenzothiazole.

3. On HPLC analysis of dilute solutions of p-benzoquinone in methanol (Whatman C8, 6% CH_3CN in water, 1 ml/min) we had observed formation of peaks attributable to 2-methoxy-p-benzoquinone, hydroquinone and 2,5-dimethoxy-p-benzoquinone. We believe that 2-methoxy-[U-^{14}C]benzoquinone is being formed and is reacting instantly with (3) to yield (4a).

4. For an analogous example see, Afanas'eva G.B., Vysokov, V.I., Pashkevich, T.K. and Chupakhin O.N.-Khim. Geterotskil Soedin. 2: 214 (1983); CA. 98: 215551q (1983).

5. Adams, R. - Organic Reactions, J. Wiley & Sons (NY), III: 240 (1946). Lin A.J. and Kasina S. - J. Heterocyclic Chem. 18: 759 (1981). Gupta R.R., Ojha K.G., Kalwania G.S., and Kumar M. - Heterocycles 14: 1145 (1980). Spiliadis A., Badic E., and Neagoe R. - Rev. Chim. 16: 89 (1965). Bhargava P.N. and Baliga B.T. - J. Ind. Chem. Soc. 35: 807 (1958). Mital R.L. and Jain S.K. - J. Chem. Soc. (C). 2148 (1969).

6. We thank Dr. P. Reider for the procedure and also for providing a sample of (11) which was made from bromovanillin.

7. The polar products were not identified.

8. We thank Dr. Y. Girard, Merck Frosst, Canada, for supplying pure samples of unlabeled compounds.